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PROLIFERATIONS OF THE EPITHELIUM INDUCED BY SUDAN III, SCHARLACH ROTH, AND PARAFFIN; AND THE EFFECTS OF ROENTGEN IRRADIATION UPON THE SAME.* †

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EVER since the cause of cancer has been a matter of consideration, experimenters have attempted, by various means, to induce cancer in animals, hoping to open the way to ascertain the etiology. Basing their operations, generally, upon some feature in the history presented by persons or animals afflicted with carcinomata, such agencies as direct trauma, heat, electric current, Roentgen irradiation, corrosive chemicals, etc., have been made use of, in efforts to produce a neoplasm of carcinomatous nature. The results of these experiments have been practically uniformly negative.

Based somewhat upon such experimentation, several theories regarding the etiology of cancer have been set forth. These, in their turn, have had the sanction and approval of pathologists in general, only to yield at last under the more diligent and painstaking investigation on the part of their supporters as well as of their opponents.

The outcome of the discussions has brought about a practical discarding of the theory of parasitic relationship to cancer, and a wavering division of pathologists between Ribbert's and Cohnheim's theories, with a few advocates of some minor theories and modifications. No phase of medical research is receiving more attention than the etiology of new growths, and none is more undetermined.

Nearly three years ago Bernard Fischer¹ gave a renewed impetus to experimental investigation into this question by reporting a series of experiments conducted by himself. It is not necessary to review the details of his operations since these are generally familiar. Suffice it to say that, following the subcutaneous injections of certain dyes, in oil, he secured a marked proliferation of epithelium which he considered to resemble the squamous-celled carcinoma in all essentials. He attributed to the dye-oil a property of attracting the epithelium from the surface into the deeper tissues. The active element he named "attraxin," and thereupon he asserted the attraxin theory.

Following Fischer's report a great many laboratory workers set out to duplicate

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† Read at the meeting of American Association of Pathologists and Bacteriologists, at Ann Arbor, April, 1908.

¹ *Münch. med. Wchnschr.*, 1906, 53, p. 2041.

and so to confirm his observations, or, failing to secure results similar to his, to challenge the accuracy of his observations and judgment.

A considerable amount of experimentation along this line has been carried out by Snow,¹ McConnell,² Helmholtz,³ and Hertzler.⁴ The last three named report work and observations warranting the acceptance of Fischer's findings, as far as the matter of epithelial proliferation is concerned, while Snow's report tended to the opposite conclusion.

Hertzler attributes the results he secured, not to an "attraxin" action on the part



FIG. 1.—Section from ear of rabbit 21 days after injection with soft paraffin. Note the hyperplastic epithelium about the hair follicles. (Snow.)

of the dye and directed toward the cells of the epithelial zone, but to "some disturbance in the chemical relationship of the different kinds of cells." He draws the general conclusion, then, that "when a chemical which has the power of combining with the acidophilic elements is injected into a tissue made up of epithelium and connective tissue, the epithelium proliferates, and invades the connective tissue, simulating the process in beginning epithelioma."

Helmholtz broadened his experiments to the extent of inducing proliferation of epithelium of mucous membranes, as well as of the skin. In one of his specimens he has demonstrated a multiple chondroma which he considers as being a consequence of the action of the injected dye.

¹ *Jour. Infect. Dis.*, 1907, 4, p. 385.

³ *Johns Hopkins Hosp. Bull.*, 1908, 18, pp. 365, 369.

² *Jour. Amer. Med. Assoc.*, 1907, 49, p. 1498. ⁴ *Jour. Amer. Med. Assoc.*, 1908, 50, p. 425.

The work in this laboratory has been limited to that done by Snow. His first set of experiments was reported in June, 1907, and in so far as agreeing with Fischer's results they were decidedly negative. Later, in work not reported, he secured a hyperplasia of epithelium under the influence of Sudan III in olive oil. In addition to this he secured a marked hyperplasia of epithelium from injections of soft paraffin in paraffin oil. He has kindly placed the results of this work at my disposal with permission to report the same herewith (Fig. 1).

From the many sets of experiments, then, it can be asserted that injection of certain dyes, as Sudan III and Scharlach Roth, in olive oil does cause a proliferation of the epithelial cells. Practical application of this fact is being made in the use of ointments containing a small percentage of these dyes, to stimulate the processes in skin grafting. Schmieden¹ reports very successful work in this line from an 8 per cent salve of Scharlach Roth. He considers the process safe, without any tendency to cancer formation, and attributes to the dye a specific chemotactic action toward the epithelial cells.²

Basing our judgments upon the work done in this laboratory, it has been maintained from the first, by the experimenters and observers here, that this produced condition does not correspond in essential details with the squamous-cell carcinoma.

At the suggestion of Dr. Warthin, and under his directions, I have undertaken to repeat Snow's experiments and, in addition, to ascertain the effects of Roentgen irradiation on this induced epithelial proliferation.³

Inasmuch as only the general effect of the X-ray was sought, i. e., whether the irradiation tends to stimulate or to inhibit the process, many of the minor details, necessary to be considered in a complete study of the question, were omitted. No exact measurements of the irradiations were made. In all cases medium tubes were used, at eight inches distance from the parts exposed, and with ten minutes' exposures. The solution injected was a saturated solution of Sudan III in pure olive oil. This solution, having been proven to have no antiseptic property in itself (report from the Department of Bacteriology), was rendered sterile by fractional sterilization, and was always injected with care regarding infection.

The injections were made as superficially as possible in the lateral external surface of rabbits' ears. The precaution to make the injections as superficially as possible was used at the suggestion of Hertz-

¹ *Centralbl. f. Chir.*, 1908, 6, p. 153.

² Carnezzì (*Gaz. degli Ospedali e delle Cliniche, Milan*, 1909, February 2, No. 14) reports very satisfactory results along the lines of Schmieden's work using 8-10 per cent Scharlach Roth salve to promote epithelial growth in the process of skin grafting.

³ My obligations are due to Professor Warthin for the original suggestion to do this piece of work, and for further instructions as the work progressed, also for assistance in securing the literature bearing upon the subject. I am indebted also to Mr. V. J. Willey of the University Laboratory of Roentgenology for assistance in his laboratory, and for permission to use his apparatus for the irradiations.

ler, who further says that Snow's failure to secure satisfactory results from his first work may be attributed to the injections being made too deep into the tissue.

At first a few ears were used to determine the separate effects of X-ray and of Sudan III.

EXPERIMENT 1: *To ascertain the effects of the X-ray alone.*—Two Belgian hares were used for this purpose. Throughout a period of 14 days ears were exposed under the conditions stated above. One ear was irradiated every second day, two daily, and one twice a day. In each case the animals were so protected by lead plates as to restrict the irradiation to the ear desired to be exposed.

The results of these experiments are simple. The effects upon the epithelium, with which alone in all of these experiments we are concerned, consist simply of an increased pigmentation of the basal cells. This pigmentation is least marked in the ear exposed every second day, and most marked in the ear exposed twice a day. The amount of pigment, however, does not seem to be dependent absolutely upon the amount of irradiation, as some ears show more increase in pigment under daily exposures than some others do from exposures twice a day. No special pains were taken to account for these differences, but it was thought that this is, perhaps, dependent upon the specific amount of pigment normally present in the skin of a given animal. The black rabbits showed a greater increase in pigmentation than the white or gray ones did. The amount of increase in pigmentation was judged by comparing sections from the irradiated ear with sections taken before the irradiation or with sections from the base of the ears which were protected by the lead plates. Beyond this pigmentation there was no change considerable with the question at hand. There was certainly no sign of hyperplasia, but rather an atrophy of the elements of the tissue.

EXPERIMENT 2: *To ascertain the effects of Sudan III-oil alone, also under the influence of Roentgen irradiation.*—Five Belgian hares were used for this purpose. Both ears of each animal were injected with Sudan III-oil. The amounts injected, the sites of the injections, and all conditions were identical in both ears of each animal. One week later the injections were repeated with the same precautions. At the end of the second week pieces were removed from all ears, fixed in formalin and in Zenker's solution, and stained in hematoxylin and eosin. During the course of the two weeks the several ears were given Roentgen irradiation at intervals of from twice a day to once every second day. In every instance the two ears of each animal were given different amounts of irradiation for sake of comparison, which was considered preferable to comparing the ears of different rabbits in any case.

The detailed findings in but three of the animals will be given here, as the other two served only as duplicates and gave the same results.

Hare 1.—Both ears were injected, according to the conditions outlined above. The left ear was not irradiated. The right ear was irradiated once every second day for two weeks.

But slight differences could be noticed between the gross appearances of the two ears during the time of treatment. Both ears thickened somewhat and presented a scaly surface. The right ear was somewhat darker than the left, but not enough to be considered at all marked.

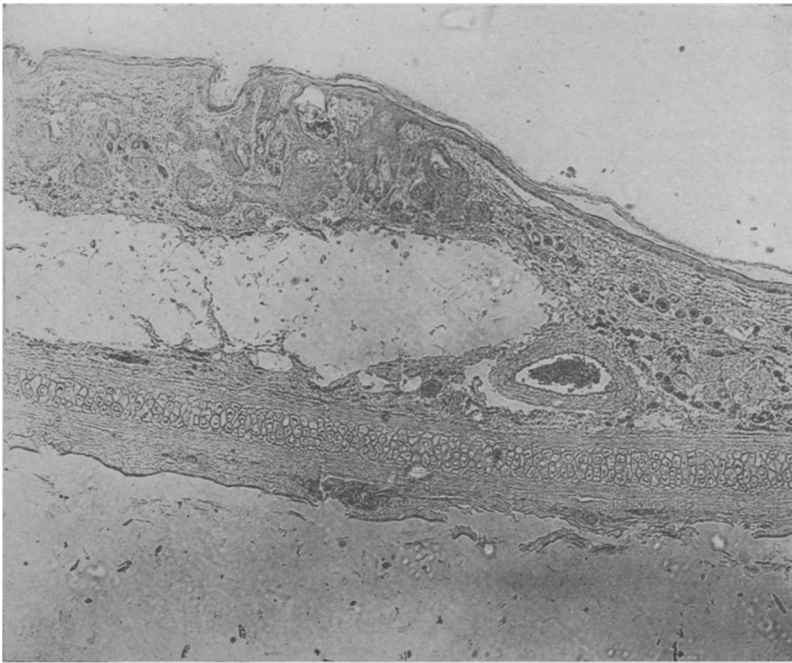


FIG. 2.—Section from ear of rabbit 14 days after injection with a saturated solution of Sudan II in olive oil. Note the extensive proliferation of epithelium showing arrangements resembling epithelial pearls and ingrowths from the surface layer of cells. These are, in fact, the masses of hyperplastic epithelium about hair follicles, which have been cut at various angles and so have this carcinomatous appearance. The portion of the figure at the right side shows the normal epithelium and hair follicles.

In this case, and to a greater degree in the next two cases, there was noted a difference in temperature in the two ears—the irradiated ear in each case feeling decidedly cool as compared with the mate.

On section these show practically the same features which have been described by several observers (Figs. 2 and 3). The most marked change from the normal picture is shown in the amount and the arrangement of the epithelium. The marginal layer of cells is increased in thickness, the cells staining practically the same as in the

normal condition. The great change in appearance is about the hair follicles. Here there is an extensive hyperplasia of epithelium, giving rise to large, some oval, some irregularly shaped, patches of cells. Most of these areas distinctly show themselves to be associated with hairs, while others, if considered in and of themselves, would give no suggestion of relation to hair follicles. It is easy to find areas, circular in outline, in which the concentric arrangement of the cells is very evident, the inner rows taking the stain less distinctly, and in all respects resembling the epithelium pearls of the squamous-

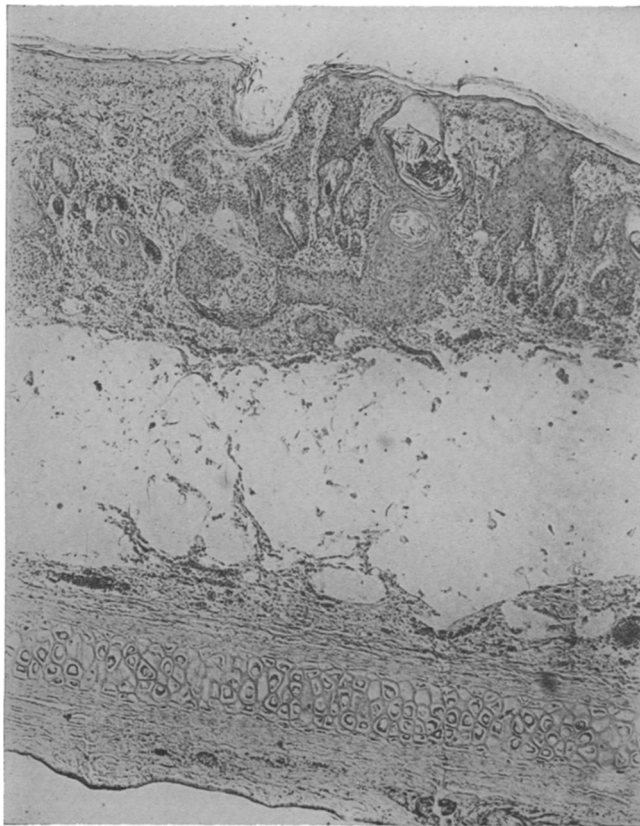


FIG. 3.—Higher magnification of section shown in Fig. 2.

cell carcinoma. Then, too, it is possible to find places where the mass of epithelial cells deep in the tissues is so connected with the surface as to appear to be a direct ingrowth of the surface zone of cells. Both of these features, which have been described as marking a close relationship between this process and that of carcinoma, can easily be accounted for as coming in all cases from the proliferation of cells about the hair follicles, if we consider the possibilities there are in this direction resulting from the cutting of sections through these areas. Serial sections easily prove the truth of this point.

This point was insisted upon by Snow, in his report cited above, as explaining the striking features of the sections offered by some workers at that time. These, as well as the cysts described, I believe are all derived from the hyperplasia of the epithelium about hair follicles.

At no place have I seen the epithelium invading the connective tissue or the cartilage, but there are places where the cartilage plate is the limit of the growth, and should such a proliferation occur immediately over one of the normal interruptions of the cartilage plate, it might extend between the two portions, and so appear to have grown into, or even entirely through, the cartilage.

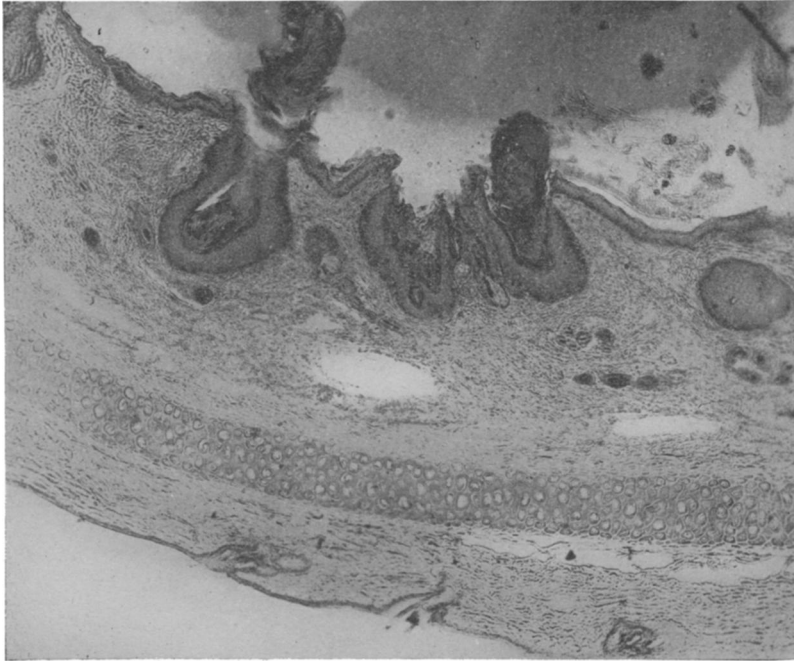


FIG. 4.—Section from ear of rabbit 14 days after injection with a saturated solution of Sudan III in olive oil, during which time the ear was irradiated daily. Note the lessened amount of epithelial hyperplasia which is localized about the hair follicles. Also note the pigmentation of the basal cells of the surface zone and the peripheral cells of the follicular masses.

The clear, generally oval, sometimes irregularly shaped spaces are oil spaces. These have no wall except the connective tissue, which is somewhat compressed and the inner layer flattened, so that it has in some places the appearance of an endothelium or of a flattened epithelium. I find no place, however, where it would be safe to say that epithelial proliferation has taken place about one of these oil spaces. These are the conditions in the left ear, which was not irradiated.

In the right ear, irradiated every second day, these same features exist, and are different from those in the left only in degree. The examination of a single picked section from each ear might leave one in doubt as to any existing difference, but after

running over a great many sections from each ear, the conclusion that there is less of cell proliferation in the irradiated ear is easily arrived at. The cells are less distinctly stained in these sections, especially about the hair follicles, than in the set just described. The irradiated ear shows the same increased pigmentation as that seen in the irradiated non-injected rabbits' ears.

Hare 2.—Both ears were injected under conditions stated above. The right ear was exposed to the X-rays daily, while the left ear was irradiated only every second day for two weeks. Sets of sections were made from the ears, as in the preceding case.

Here we have all the features given in the sections from the first animal, with,

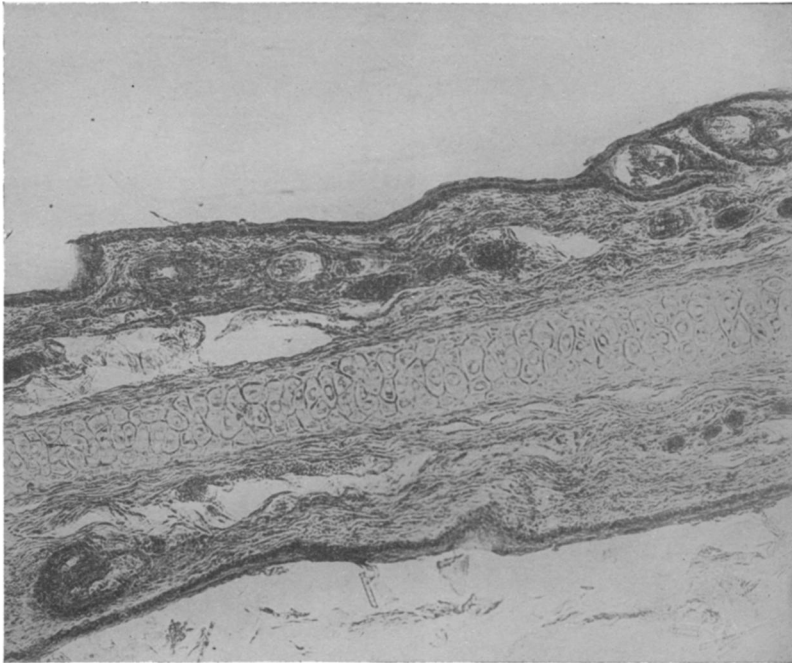


FIG. 5.—Section from ear of rabbit 14 days after injection with a saturated solution of Sudan III in olive oil, during which time the ear was irradiated twice each day. Note the absence of epithelial proliferation. Also note the intense pigmentation.

however, a more marked diminution in epithelial growth (Fig. 4). There are patches about the hair follicles, as before, but it does not give one the impression of having developed so abundantly as in the other cases. The line of pigmentation is distinct in the sections from the ear that was irradiated daily, and is continuous around all the projections and isolated areas of epithelium. This distribution of pigment does not agree with that seen in epitheliomata after treatment with X-rays, inasmuch as in irradiated carcinomata of the skin pigment is formed only in the rete of normal skin. The series of sections show that there is less proliferation in the right ear than in the left.

Hare 3.—Both ears were injected as in previous cases. The right ear was exposed

to the X-rays daily, and the left ear was irradiated twice each day for two weeks. The sections in this case show much less of proliferation in both ears. In the right ear, irradiated daily, there are thickenings about the hair follicles, but not extending deep into the tissues, and showing but few and small isolated areas. These are everywhere bounded by the heavily pigmented layer of cells. In the left ear, irradiated twice a day, the amount and distribution of epithelium differ but little from that in the normal, untreated ear (Fig. 5). There are a few slight thickenings about the hair follicles, but no outgrowths or isolated patches. The basement layer of cells is very heavily pigmented. The oil spaces are very numerous in these sections, showing that the inactivity is not due to a lack of Sudan III-oil.

CONCLUSIONS FROM THE FOREGOING EXPERIMENTS.

1. Sudan III-oil may induce a proliferation of epithelium. This proliferation in no sense simulates carcinoma, but is merely a simple hyperplasia of the epithelium about the hair follicles, and in single sections may be mistaken for an infiltration. There is, however, no true infiltration of connective tissue or of cartilage; the normal relationships and characteristics of the cell-layers of the epidermis are preserved; and the hyperplasia can in no sense be regarded as analogous to a neoplastic epithelial proliferation.

2. Whether this hyperplasia is due to the Sudan III or to the olive oil is a question. Snow's securing a hyperplasia from the use of paraffin oil alone makes this an open question, and does not bear out Hertzler's explanation of the proliferation as being due to the removal of the restraining influence upon the epithelium by the combination of the injected material with the connective tissue.

3. Roentgen irradiation inhibits this proliferation, and the irradiation is more efficient when frequently applied.

This last conclusion is in accord with what one would expect after reading the literature bearing upon the effects of Roentgen irradiation upon the tissues, especially upon the elements of the skin. The complete literature upon this subject has been assembled by Professor Warthin.¹ It is not thought necessary to reproduce at all extensively that report here. In practically every case in which an active process was under consideration, it was shown that the X-rays have a restraining influence. As somewhat closely related to this set of experiments, the work of Oudin, Barthélemy, and Darier² may be cited.

¹ *Internat. Clin.*, 1906, 15, s. iv, p. 243; *Physician and Surgeon*, 1907, 29, p. 1.

² *Monatshefte f. prakt. Dermatol.*, 25, p. 417.

These experimenters secured in guinea-pigs a marked atrophy of the hair follicles from exposure to X-rays. Later, Jutassy¹ secured the same results in irradiated rabbits. The use of conclusions from such experiments as these to strengthen the policy of using X-ray in the treatment of epitheliomata is more or less justifiable, inasmuch as in both conditions the prominent feature is a proliferation of epithelium. Perhaps by continued work along this line, which affords an excellent means for control experimentation, the most efficient strengths and



FIG. 6.—Section from ear of rabbit 14 days after injection with paraffin oil. Note the inflammatory reaction and the hyperplastic hair follicles.

conditions of irradiation can be more accurately determined and so better results secured.

Two additional experiments were suggested by the foregoing.

EXPERIMENT 3: *An attempt to reproduce Snow's results of hyperplasia under the influence of paraffin oil alone, also of olive oil alone.*—Three Belgian hares were used for this purpose. One ear of each animal was injected with paraffin oil, and the other ear injected with olive oil. Within a few days the ears thickened and scaled somewhat.

¹ *Fortschr. a. d. Gebiete der Roentgenstrahlen*, 1899.

The injections were repeated at the end of a week. At the end of the second week pieces were removed from all ears, and examined in the usual way. All of the specimens show a rather marked reaction toward the foreign substances. The vessels show congestion, and within the tissues are many phagocytes. In the ears injected with paraffin oil there is shown a hyperplasia of epithelium about the hair follicles, as compared with other ears of the same animals (Fig. 6). No hyperplasia was secured in the tissues injected with olive oil.

This experiment tends to show that the condition produced by use of the Sudan III-oil was due to the Sudan III, or possibly to the

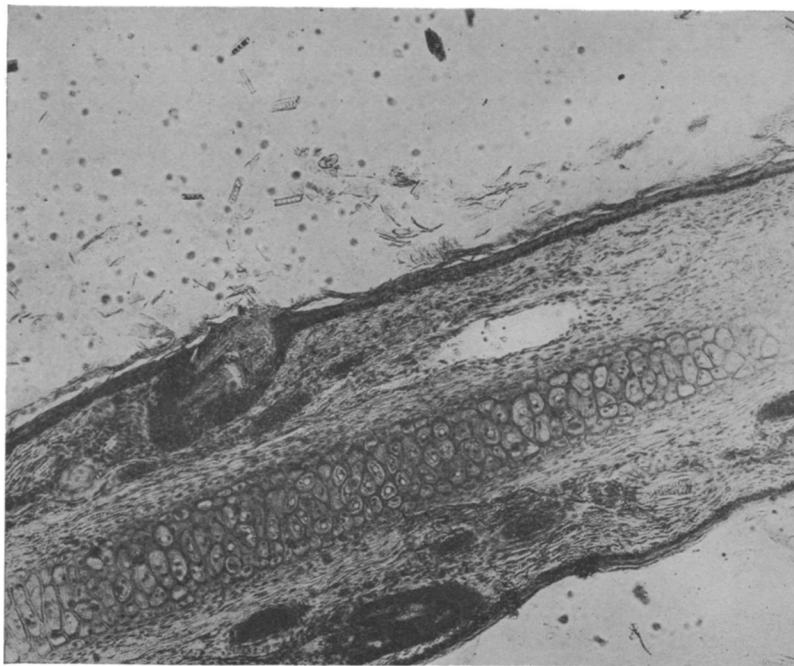


FIG. 7.—Section from ear of rabbit which was irradiated twice a day for 14 days and then injected with a saturated solution of Sudan III in olive oil. The section represents the condition 14 days after the injection. Note the resemblance of this section to the one shown in Fig. 5. Note the intense pigmentation and lack of epithelial hyperplasia.

combination of dye and oil, but not due to the olive oil alone. This may be explained by the more irritant property of the Sudan III and of the paraffin oil, as compared with the bland olive oil. It also shows that this condition does not depend upon the presence of a substance which combines with the tissue elements. And, furthermore, it serves to establish the work done by Snow in this laboratory.

EXPERIMENT 4: *To ascertain the effects of Sudan III-oil after Roentgen irradiation.*—Two Belgian hares were used. One ear of each animal was irradiated daily, and the other ear of each twice a day (two days only once) for two weeks. Then all the ears were injected with Sudan III-oil as in the previous cases, and the irradiations suspended. The tissues (Fig. 7), at the end of two weeks, showed the pigmentation characteristic of the X-ray effect, the oil spaces, some inflammatory reaction, but nothing that could be construed as an increased growth of the epithelium.

This experiment gives us further evidence of the restraining influence of the Roentgen rays, also the persistence of this inhibitory effect after the suspension of the exposures.

NOTE.—Since this work was reported at the meeting of the American Association of Pathologists and Bacteriologists, April, 1908, Werner¹ has conducted a set of experiments with reference to the effects of Scharlach Roth injections into mouse tumors. He arrives at the same conclusion, i. e., that this proliferation is simply the result of irritation and not due to any "attraxin" principle on the part of the dye. This we consider as a confirmation of the conclusion arrived at in this laboratory by the work of Snow and myself, that all of the proliferation obtained from injections of Sudan III, Scharlach Roth, paraffin, and paraffin-oil is purely an irritation hyperplasia having no neoplastic characteristics.

¹ *Munch. med. Wchnschr.*, 1908, 55, p. 2265.